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(72) Inventors:

- **Bernard-Pierrot, Isabelle**  
**94130, NOGENT SUR MARNE (FR)**
- **Radvanyi, François**  
**92260, FONTENAY AUX ROSES (FR)**
- **Allory, Yves**  
**75011, PARIS (FR)**
- **Stransky, Nicolas**  
**94270, LE KREMLIN BICÊTRE (FR)**

(71) Applicants:

- **Institut Curie**  
**75248 Paris Cedex 05 (FR)**
- **Centre National de la Recherche Scientifique**  
**75016 Paris (FR)**
- **Assistance Publique - Hôpitaux de Paris**  
**75004 Paris (FR)**
- **Universite Paris XII**  
**94010 Creteil (FR)**

(74) Representative: **Gallois, Valérie et al**  
**Cabinet BECKER & ASSOCIES**  
**25, rue Louis Le Grand**  
**75002 Paris (FR)**

(54) **Tyrosine kinase receptor Tyro3 as a therapeutic target in the treatment of a bladder tumor**

(57) The present invention concerns new methods for treating bladder cancer by using TYRO3 inhibitors and methods for identifying new molecules of interest for treating bladder cancer.

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**Description****FIELD OF THE INVENTION**

**[0001]** The present invention relates to the field of medicine, in particular to the treatment of bladder cancer. It relates to new methods of treatment of bladder cancer and to methods of screening of molecules useful in the treatment of bladder cancer.

**BACKGROUND OF THE INVENTION**

**[0002]** Bladder cancer is the fifth cancer in term of incidence. It can appear as superficial lesions restricted to the urothelium (Ta and carcinoma in situ (CIS)) or to the lamina propria (T1) or as muscle invasive lesions (T2-T4). Two different pathways of tumour progression have been so far described in bladder cancer, the Ta pathway and the CIS pathway. Ta tumours which constitute 50% of bladder tumours at first presentation are superficial papillary tumour usually of low grade which do not invade the basal membrane. Carcinoma-in-situ (CIS) are also superficial tumour which do not invade the basal membrane but are always of high grade. Ta tumours, despite surgical resection associated or not with BCG (Bacillus Calmette-Guerin) therapy, often recur but rarely progress to muscle invasive disease (T2-T4), whereas CIS often progress to T2-T4 tumors. Concerning muscle invasive bladder carcinomas, the standard treatment is cystectomy. Despite this radical treatment, muscle invasive bladder carcinoma remains a deadly disease for most patients.

**[0003]** Up to now, even if many recurrent chromosomal alterations have been described in bladder cancer, only few genes have been demonstrated to be implicated in tumor progression (p53, CDKN2A, RB1, E2F3, FGFR3).

**[0004]** Accordingly, there is a significant need for an appropriate bladder tumor treatment, in particular for new and more effective therapeutic agents.

**SUMMARY OF THE INVENTION**

**[0005]** The present invention provides new therapeutic agents for treating bladder tumor.

**[0006]** In a first aspect, the present invention concerns an inhibitor of TYR03 tyrosine kinase for the treatment of a bladder tumor.

**[0007]** The present invention also concerns a pharmaceutical composition comprising an inhibitor of TYR03 tyrosine kinase and a pharmaceutically acceptable carrier/excipient for the treatment of a bladder tumor.

**[0008]** The TYR03 tyrosine kinase inhibitor is preferably selected from the group consisting of a small molecule inhibiting the TYR03 tyrosine kinase activity, an antibody directed against the extracellular domain of TYRO3, a nucleic acid molecule interfering specifically with TYR03 expression, a dominant negative receptor presenting a kinase dead domain and a TYR03 soluble bait.

**[0009]** In a particular embodiment, the TYR03 tyrosine kinase inhibitor is a RNAi, an antisense nucleic acid or a ribozyme interfering specifically with TYR03 expression.

**[0010]** In a preferred embodiment, the inhibitor is a siRNA, in particular siRNA comprising a sequence of SEQ ID No. 1.

**[0011]** In another embodiment, the inhibitor is a TYR03 soluble bait, in particular a recombinant TYR03 receptor constituted of, at least, the extracellular domain of the receptor

**[0012]** In one embodiment, the TYR03 tyrosine kinase inhibitor is used in combination with another active ingredient, in particular a bladder tumor treatment.

**[0013]** In another aspect, the present invention concerns a method for screening or identifying a molecule suitable for treating a bladder tumor comprises (i) contacting candidate molecules with TYR03 receptor, and (ii) selecting molecules having the ability to bind to TYR03 receptor and/or to compete with and/or for a ligand of TYR03 receptor and/or to decrease the phosphorylation of the TYR03 substrates or the TYR03 autophosphorylation.

**[0014]** The present invention also concerns a method for screening or identifying a molecule suitable for treating a bladder tumor comprising (i) contacting candidate molecules with cells expressing TYR03 receptor, and (ii) selecting molecules having the ability to bind to TYR03 receptor and/or to compete with and/or for a ligand of TYR03 receptor and/or to decrease the TYR03 gene expression and/or to decrease the phosphorylation of the TYR03 substrates or the TYR03 autophosphorylation.

**[0015]** These methods for screening or identifying a molecule suitable for treating a bladder tumor can optionally further comprise administering *in vitro* selected molecule in a bladder tumor non human animal model and analyzing the effect on the disease progression.

**[0016]** The present invention further concerns a method for treating a bladder tumor in a subject comprising administering a therapeutically efficient amount of a TYR03 tyrosine kinase inhibitor to the subject.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0017]

Figure 1 shows TYRO3 expression in bladder tumors and bladder cancer cell lines. mRNA expression levels in human bladder cancer tumors were assessed using Affymetrix U95A DNA microarray (Figure 1A). The difference in expression between different groups were compared using an ANOVA test (Figure 1B)

Figure 2 shows GAS6 expression in bladder tumors and bladder cancer cell lines. mRNA expression levels in human bladder cancer tumors were assessed using Affymetrix U95A DNA microarray (Figure 2A) and the difference in expression between different groups were compared using an ANOVA test (Figure 2B)

Figure 3 shows TYRO3 and GAS6 mRNA expression levels in human bladder cancer cell lines T24, MGH-U3, RT4, KK47, TCCSUP, EJ138, J82 and RT112 and in NHU normal urothelium derived cell line assessed by Q-RT-PCR.

Figure 4 shows the efficiency of TYRO3 silencing in MGH-U3 cells. Cells were transfected with 50 nM siRNA (siRNA anti-TYRO3 (SEQ ID No. 1) or control siRNA (scramble, SEQ ID No. 2)) and the efficiency of TYRO3 silencing was assessed 72 hours after transfection by western-blot.

Figure 5 shows a graph representing the effect of TYRO3 knockdown on bladder cancer cell viability. After transfection as described in the legend of figure 4, cells were treated with trypsin 72 hours after transfection, stained with trypan blue and viable cells were counted in triplicate, using a Malassez hematocytometer. Results are the means  $\pm$  SD of two independent experiments carried out in triplicate.

Figure 6 shows the effect of TYRO3 antibody on bladder cancer cell viability. Cells were incubated 72 h in presence of various concentrations of a polyclonal antibody directed against the extra-cellular domain of TYRO3 (goat Anti-Rse (N-18), Santa-Cruz biotechnology) and cell viability was measured by MTT assay.

Figure 7 shows the effect of a recombinant soluble TYRO3 receptor on cell viability. The extra-cellular domain of TYRO3 (421 aa) composed of two Ig like domains (aa 1-220) and two fibronectin III domain (aa 220-421) was produced in bacteria and purified. Cells were incubated 72 h in presence of various amounts of this soluble receptor and cell viability was measured by MTT incorporation.

Figure 8 shows the results of a TUNEL assay on transfected bladder cancer cells.  $3 \times 10^4$  cells per well were seeded on a glass slide in a 24-well plate and transfected with 50 nM siRNA. DNA fragmentation was evaluated 72 hours after transfection, using a TUNEL (deoxynucleotidyl transferase (Tdt)-mediated nick-end labeling) assay detection Kit (Roche Diagnostic, Meylan, France) according to the manufacturer's instructions. The inventors analyzed 600 cells under a light microscope, determining the proportion of labeled cells.

Figure 9 shows cell cycle analysis by flow-cytometry 72 h post siRNA transfection. Results were analyzed using Fisher test, \*\*\*  $p < 0.001$ , \*  $0.01 < p < 0.05$

Figure 10 shows a graph representing the effect of TYRO3 knockdown on anchorage-independent colony formation.  $2 \cdot 10^4$  50 nM siRNA-transfected cells in DMEM supplemented with 10% FCS and 0.3% agar, were added to triplicate wells containing medium and 0.8% agar on 12-well plates. The plates were incubated for two weeks and colonies with diameters greater than 50  $\mu$ m were scored as positive, using a phase-contrast microscope equipped with a measuring grid. Results are the means  $\pm$  SD of two independent experiments carried out in triplicate.

Figure 11 shows the effect of TYRO3 siRNA on the growth of xenografted J82 tumors. Tumor-bearing mice were treated three times a week by intraperitoneal injection of 4  $\mu$ g siRNA (control or TYRO3) (6 mice and 12 tumors per group) (The first injection corresponds to day 0). Tumor volume variations are represented on the graph of Figure 11A. (Wilcoxon rank sum test: \*,  $0.05 < p < 0.01$ ; \*\*,  $0.01 < p < 0.001$ ; \*\*\*,  $p < 0.001$ ) Inset are pictures of representative tumors observed at the end of the treatment. The upper line tumors are those of control siRNA treated mice. The lower line tumors are those of TYRO3 siRNA treated mice. Tumors were weighted at the end of the experiment (Figure 11B).

Figure 12 shows the effect of TYRO3 siRNA on the growth of xenografted J82 tumors. Tumor-bearing mice were treated three times a week by intraperitoneal injection of 4  $\mu$ g siRNA (control or TYRO3) (6 mice and 12 tumors per group) (The first injection corresponds to day 0). Tumor volume variations are represented on the graph of Figure 12A. (Wilcoxon rank sum test: \*,  $0.05 < p < 0.01$ , \*\*,  $0.01 < p < 0.001$ , \*\*\*,  $p < 0.001$ ) Inset are pictures of representative tumors observed at the end of the treatment. The upper line tumors are those of TYRO3 siRNA treated mice. The middle line tumors are those of control siRNA treated mice. The lower line tumors are those of PBS treated mice. Tumors were weighted at the end of the experiment (Figure 12B).

Figure 13 is a graph showing TYRO3 mRNA levels for MGH-U3 xenografts, 3 days after the last siRNA injection, divided by TBP (TATA binding protein) mRNA levels  $\pm$  SD in treated and control tumors, as assessed by Q-RT-PCR.

## DETAILED DESCRIPTION OF THE INVENTION

[0018] By analysing the transcriptome in a series of 80 bladder carcinomas, 5 normal bladder urothelium and 10

bladder tumour cell lines, the inventors have :

- identified a tyrosine kinase receptor TYR03 overexpressed in almost 70% of bladder carcinoma as compared to normal urothelium samples, this over-expression being independent of stage and grade;
- noticed that one of the TYR03 ligands, GAS6, is also over-expressed in invasive carcinoma as compared to normal urothelium and superficial tumors; and,
- functionally demonstrated the importance of TYR03 in bladder tumors cell survival.

**[0019]** QPCR analysis validated Affymetrix transcriptomic data and showed hence an over-expression of TYR03 in most bladder tumor samples. *In situ* hybridization demonstrated that TYR03 is expressed by bladder tumor epithelial cells whereas GAS6 is mainly expressed by stromal cells. Functional studies of TYR03 in four bladder tumor cell lines (two expressing only TYR03 and two expressing both TYR03 and GAS6) using siRNA to knock down gene expression showed that TYR03 was necessary for bladder cancer cell survival both *in vitro* and *in vivo*. Indeed, inactivation of TYR03 1) inhibits cell survival by inducing cell apoptosis; 2) inhibits anchorage independent growth demonstrating that TYR03 regulates cell survival of clonogenic cells; 3) inhibits growth of bladder tumour cells xenografted in nude mice and even more reduces the size of tumor. Interestingly, the inventors observed the same *in vitro* effect through TYR03 activity inhibition using a polyclonal anti-TYR03 antibody directed against its extracellular domain or using a recombinant TYR03 soluble receptor (constituted of the extracellular domain of the receptor).

**[0020]** Accordingly, TYR03 has been demonstrated by the inventors to be a tyrosine kinase overexpressed in most bladder tumors and to induce tumor cell survival. Therefore, the present invention provides a new interesting therapeutic target in bladder cancer.

**[0021]** Up to now, TYR03 has been described to be over-expressed or co-expressed with its ligand in few human tumor types (uterine liomyoma (Sun et al., 2003a), uterine endometrial cancers and ovarian endometriose (Sun et al., 2002, Sun et al., 2003b), lung carcinoma (Wimmel et al., 1999)) but its role in tumor progression and especially in tumor cell survival has never been suggested nor demonstrated.

**[0022]** Its oncogenic role has been suggested since its expression transformed Rat-2 fibroblasts and RatB1 fibroblast (lai et al., 2004; Taylor et al., 1995). Furthermore, these oncogenic properties have also been suggested as a hybrid receptor constituted of the extracellular of the EGF receptor and the intracellular part of TYR03 can transform NIH3T3 cells in presence of EGF (lan et al., 2000).

**[0023]** However, the potential oncogenic role of TYR03 does not disclose nor suggest the role of this receptor in the tumor cell survival.

**[0024]** Recently, a withdrawn patent application (W02005000207) by Kiener et al. suggests that TYR03 is the receptor of PCDGF (PC cell derived growth factor). Since PCDGF is overexpressed in different cancers, this document suggests that inhibiting PCDGF binding to TYR03 could be a therapeutic approach in several cancers overexpressing PCDGF. However, this document does not contain any data supporting their approach. Indeed, there is no data demonstrating that TYR03 is the receptor of PCDGF and that a molecule inhibiting the potential binding of PCDGF to TYR03 may have an effect of cancer cells.

**[0025]** Accordingly, for the first time, the role of TYRO3 in the tumor cell survival has been described and proved and this role provides a new means to treat an existing bladder tumor.

**[0026]** Tyrosine kinase receptors are composed of an extracellular domain, which is able to bind a specific ligand, a transmembrane domain, and an intracellular catalytic domain, which is able to bind and phosphorylate selected intracellular substrates. Binding of a ligand to the extracellular region causes a series of structural rearrangements in the tyrosine kinase receptor that lead to its enzymatic activation triggering a cascade of events through phosphorylation of intracellular proteins that ultimately transduces the extracellular signal to the nucleus, causing changes in gene expression.

**[0027]** TYR03 tyrosine kinase is a member of the AXL/Ufo/Mer tyrosine kinase receptor family. TYR03 is also known as BYK, Brt, Dtk, Rse, Sky or Tif. Gas6 (growth arrest specific gene-6) and protein S have been described to activate TYR03 tyrosine kinase activity.

**[0028]** The polynucleotide and amino acid sequences are well-known in the art. Reference sequences are Genbank Accession Nos MN\_006293.2 and NP\_006284.2, respectively. The reference entry for human TYR03 in the transcriptome database UniGene is Hs.381282.

**[0029]** In the present invention, an "inhibitor of TYR03 tyrosine kinase" is a molecule which inhibits or reduces the activity of the TYR03 receptor. Thus, the inhibitor induces the suppression or the reduction of the transmission of extracellular signals into the cell through the TYR03 receptor.

**[0030]** The activity of TYR03 tyrosine kinase activity can be easily assayed by any method known in the art. A first assay can be the determination of the ability of the inhibitor to bind the TYR03 receptor. A second assay can be the determination of the ability of the inhibitor to compete with a ligand of the TYR03 receptor for the binding of this receptor or of this ligand. A third assay can be the determination of the ability of the inhibitor to decrease the TYR03 expression

level. A fourth assay can be the determination of the ability of the inhibitor to decrease the phosphorylation of the TYR03 substrates or the TYR03 autophosphorylation. These different methods are described below in this document and can be combined.

**[0031]** The inhibition can be due to the binding of a molecule on the extracellular domain of the receptor. In this case, the inhibitor can be an antagonist which binds to the ligand binding domain or another domain of the receptor, or a molecule which modifies the activity of the receptor by steric hindrance or modification. This inhibitor can be, for instance, a small molecule, an aptamer or an antibody directed against the extracellular domain of the receptor. The inhibitory activity can be determined through a binding assay, a competitive binding assay or a phosphorylation assay.

**[0032]** The inhibition can also be due to the reduction or suppression of the expression of the gene coding for the receptor, for example by using specific RNAi, antisense or ribozyme, which induces a decrease of the number of receptors at the cell surface and thus a reduction of the extracellular signal transmission. The inhibitory activity can be assayed through the measure of the expression level of TYR03, at the protein level or RNA level. The inhibitory activity can also be assayed through the phosphorylation of TYR03 or TYR03 substrate.

**[0033]** The use of baits which bind ligands of the TYR03 receptor can also induce reduction or suppression of the activity of this receptor by competition for these ligands. Indeed, these baits trap ligands of TYR03 and, consequently, decrease the concentration of available ligands for TYR03 activation. These baits can be disposed in the membrane such as dominant negative receptors or in the extracellular fluid such as soluble receptor. The inhibitory activity can be determined through a competition assay in order to determine the decrease of binding between the functional TYR03 receptor and its ligand. The inhibitory activity can also be assayed through the phosphorylation of TYR03 or TYR03 substrate.

**[0034]** In preferred embodiments of the present invention, the inhibitor of TYR03 tyrosine kinase is preferably selected from the group consisting of a small molecule inhibiting the tyrosine kinase activity, an antibody directed against the extracellular domain of TYR03, a nucleic acid molecule interfering specifically with TYR03 expression, a dominant negative receptor presenting a kinase dead domain and a TYR03 soluble bait.

**[0035]** As used herein, the term "small molecule inhibiting the tyrosine kinase activity" refers to small molecule that can be an organic or inorganic compound, usually less than 1000 daltons, with the ability to inhibit or reduce the activity of the TYR03 tyrosine kinase. This small molecule can be derived from any known organism (including, but not limited to, animals, plants, bacteria, fungi and viruses) or from a library of synthetic molecules. Small molecules inhibiting the TYR03 tyrosine kinase activity can be identified with the method further described in this document.

**[0036]** In a particular embodiment, this molecule is selected from the group consisting of CHIR-258/TKI-258 (Novartis Pharmaceuticals), CI-1033 (Pfizer Pharmaceuticals), EKB-569 (Wyeth Pharmaceuticals), Erlotinib/ Tarceva® (OSI Pharmaceuticals), MLN-8054 (Millennium Pharmaceuticals), staurosporine (Calbiochem), SU-14813 (Pfizer Pharmaceuticals), Sunitinib/sutent® (Pfizer Pharmaceuticals) and ZD-6474 (AstraZeneca Pharmaceuticals) (see also Karaman et al., 2008).

**[0037]** As used herein, the term "antibody" is intended to refer broadly to any immunologic binding agent such as IgG, IgM, IgA, IgD and IgE, and humanized or chimeric antibody. In certain embodiments, IgG and/or IgM are preferred because they are the most common antibodies in the physiological situation and they are most easily manufactured. The term "antibody" is used to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')<sub>2</sub>, single domain antibodies (DABs), Fv, scFv (single chain Fv), and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means for preparing and characterizing antibodies are also well known in the art (See, e.g., Harlow and Lane, 1988).

**[0038]** A "humanized" antibody is an antibody in which the constant and variable framework region of one or more human immunoglobulins is fused with the binding region, e.g. the CDR, of an animal immunoglobulin. "Humanized" antibodies contemplated in the present invention are chimeric antibodies from mouse, rat, or other species, bearing human constant and/or variable region domains, bispecific antibodies, recombinant and engineered antibodies and fragments thereof. Such humanized antibodies are designed to maintain the binding specificity of the non-human antibody from which the binding regions are derived, but to avoid an immune reaction against the non-human antibody.

**[0039]** A "chimeric" antibody is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

**[0040]** Particularly, the term "antibody against the extracellular domain of TYR03" designates an antibody as described above which is able to bind to the extracellular domain of the TYR03 tyrosine kinase receptor and to block or reduce its activity. This inhibition can be due to steric hindrance or modification which prevents ligand binding.

**[0041]** In a preferred embodiment, the antibody directed against the extracellular domain of TYR03 is an Anti-Rse (N-18) antibody (Santa-Cruz biotechnology).

**[0042]** As used herein, a "dominant negative receptor presenting a kinase dead domain" is a receptor which is able

to bind to a ligand but is defective for the transmission of the signal. Consequently, the over-expression of a dominant-negative receptor affects receptor signalling by blocking signal transduction. The presence of such dominant negative receptor at the cell surface induces a competition for ligand decreasing the amount of available ligand for the active receptor and thus preventing the activation of this receptor. In the present invention, the dominant negative receptor

**[0043]** As used herein, the term "TYR03 soluble bait" designates an extracellular molecule which is able to bind to a TYR03 ligand. This soluble bait can be constituted of any peptide which has the ability to bind a ligand of the TYR03 receptor.

**[0044]** In one embodiment, the TYR03 soluble bait is a recombinant TYR03 receptor constituted of the extracellular domain of the receptor. In a preferred embodiment, the TYR03 soluble bait is the entire extracellular domain of TYR03 receptor. The extra-cellular domain of TYR03 (421 aa) is composed of two Ig like domains (aa 1-220) and two fibronectin III domains (aa 220-421). In another embodiment, the TYR03 soluble bait is a recombinant TYR03 receptor constituted of one or two Ig like domains or of one or two fibronectin III domains. If necessary, the TYR03 receptor domains may be coupled with a Fc Fragment to stabilize the receptor.

**[0045]** In a particular embodiment, the TYR03 soluble bait is able to bind to Gas6 and/or protein S.

**[0046]** The inhibitors of TYR03 tyrosine kinase of the invention may also be nucleic acid molecules. The term "nucleic acid molecule" includes, but is not limited to, RNAi, antisense and ribozyme molecules.

**[0047]** In the present invention, a "nucleic acid molecule specifically interfering with TYR03 expression" is a nucleic acid molecule which is able to reduce or to suppress the expression of gene coding for TYR03 receptor, in a specific way.

**[0048]** The term "RNAi" or "interfering RNA" means any RNA which is capable of down-regulating the expression of the targeted protein. It encompasses small interfering RNA (siRNA), double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), micro-RNA (miRNA), and short hairpin RNA (shRNA) molecules. RNA interference, designate a phenomenon by which dsRNA specifically suppresses expression of a target gene at post-translational level. In normal conditions, RNA interference is initiated by double-stranded RNA molecules (dsRNA) of several thousands of base pair length. In vivo, dsRNA introduced into a cell is cleaved into a mixture of short dsRNA molecules called siRNA. The enzyme that catalyzes the cleavage, Dicer, is an endo-RNase that contains RNase III domains (Bernstein, Caudy et al. 2001). In mammalian cells, the siRNAs produced by Dicer are 21-23 bp in length, with a 19 or 20 nucleotides duplex sequence, two-nucleotide 3' overhangs and 5'-triphosphate extremities (Zamore, Tuschl et al. 2000; Elbashir, Lendeckel et al. 2001; Elbashir, Martinez et al. 2001).

**[0049]** A number of patents and patent applications have described, in general terms, the use of siRNA molecules to inhibit gene expression, for example, WO 99/32619, US 20040053876, US 20040102408 and WO 2004/007718.

**[0050]** siRNA are usually designed against a region 50-100 nucleotides downstream the translation initiator codon, whereas 5'UTR (untranslated region) and 3'UTR are usually avoided. The chosen siRNA target sequence should be subjected to a BLAST search against EST database to ensure that the only desired gene is targeted. Various products are commercially available to aid in the preparation and use of siRNA.

**[0051]** In a preferred embodiment, the RNAi molecule is a siRNA of at least about 15-50 nucleotides in length, preferably about 20-30 base nucleotides, preferably about 20-25 nucleotides in length.

**[0052]** In a particular embodiment, the siRNA molecule comprises the sequence of SEQ ID No. 1.

**[0053]** RNAi can comprise naturally occurring RNA, synthetic RNA, or recombinantly produced RNA, as well as altered RNA that differs from naturally-occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end of the molecule or to one or more internal nucleotides of the RNAi, including modifications that make the RNAi resistant to nuclease digestion.

**[0054]** RNAi may be administered in free (naked) form or by the use of delivery systems that enhance stability and/or targeting, e.g., liposomes, or incorporated into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, bioadhesive microspheres, or proteinaceous vectors (WO 00/53722), or in combination with a cationic peptide (US 2007275923). They may also be administered in the form of their precursors or encoding DNAs.

**[0055]** Antisense nucleic acid can also be used to down-regulate the expression of the TYR03 receptor. The antisense nucleic acid can be complementary to all or part of a sense nucleic acid encoding a TYR03 receptor polypeptide e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence, and it thought to interfere with the translation of the target mRNA

**[0056]** In a preferred embodiment, the antisense nucleic acid is a RNA molecule complementary to a target mRNA encoding a TYR03 receptor polypeptide.

**[0057]** An antisense nucleic acid can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. Particularly, antisense RNA molecules are usually 18-50 nucleotides in length.

**[0058]** An antisense nucleic acid for use in the method of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. Particularly, antisense RNA can be chemically synthesized, produced by *in vitro* transcription from linear (e.g. PCR products) or circular templates (e.g., viral or non-

viral vectors), or produced by *in vivo* transcription from viral or non-viral vectors.

**[0059]** Antisense nucleic acid may be modified to have enhanced stability, nuclease resistance, target specificity and improved pharmacological properties. For example, antisense nucleic acid may include modified nucleotides designed to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides.

**[0060]** Ribozyme molecules can also be used to decrease levels of functional TYR03 tyrosine kinase. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes can be used to catalytically cleave mRNA transcripts to thereby inhibit translation of the protein encoded by the mRNA. Ribozyme molecules specific for functional TYR03 tyrosine kinase can be designed, produced, and administered by methods commonly known to the art (see e.g., Fanning and Symonds, 2006, reviewing therapeutic use of hammerhead ribozymes and small hairpin RNA).

**[0061]** By "bladder tumor" is intended herein urinary bladder tumor, bladder cancer or urinary bladder cancer, and bladder neoplasm or urinary bladder neoplasm. A bladder tumor can be a bladder carcinoma or a bladder adenoma. The most common staging system for bladder tumors is the TNM (tumor, node, metastasis) system. This staging system takes into account how deep the tumor has grown into the bladder, whether there is cancer in the lymph nodes and whether the cancer has spread to any other part of the body. In a preferred embodiment, the bladder tumor is a bladder carcinoma. In a preferred embodiment, the bladder carcinoma to be treated is a T stage. In addition, the bladder carcinomas of T stage can have sub-stages:

CIS - very early cancer cells are detected only in the innermost layer of the bladder lining;

Ta - the cancer is just in the innermost layer of the bladder lining;

T1 - the cancer has started to grow into the connective tissue beneath the bladder lining;

T2 - the cancer has grown through the connective tissue into the muscle;

T2a - the cancer has grown into the superficial muscle;

T2b - the cancer has grown into the deeper muscle;

T3 - the cancer has grown through the muscle into the fat layer;

T3a - the cancer in the fat layer can only be seen under a microscope;

T3b - the cancer in the fat layer can be seen on tests, or felt by the physician;

T4 - the cancer has spread outside the bladder;

T4a - the cancer has spread to the prostate, womb or vagina;

T4b - the cancer has spread to the wall of the pelvis and abdomen.

**[0062]** Accordingly, the bladder tumor or carcinoma that can be treated by the present invention can be superficial (Ta, T1) or invasive (T2 to T4). In a particular embodiment, the bladder carcinoma that can be treated by the present invention can be any and all T sub-stages.

**[0063]** In a preferred embodiment, a sample from the subject to be treated, in particular a bladder tumor sample, is assayed for the overexpression of TYR03. Accordingly, the treatment with the TYR03 inhibitor is more particularly appropriate for a subject having a bladder tumor overexpressing TYR03. The TYR03 expression can be assayed by quantitative RT-PCR or using any method known by the man skilled in the art. The TYR03 expression in the tumor tissue should be compared to the expression in normal proliferative cell lines, preferably to normal cells providing from the same tissue than the tumor.

**[0064]** As used herein, the term "treatment" of a disease refers to any act intended to extend life span of patients such as therapy and retardation of the disease. The treatment can be designed to eradicate the tumor, to stop the progression of the tumor, to prevent the occurrence of metastasis, to promote the regression of the tumor and/or to prevent muscle invasion of cancer. The patient to treat is any mammal, preferably a human being.

**[0065]** The treatment of bladder tumor with pharmaceutical composition according to the invention can be associated with other therapy such as surgery, radiation therapy or other chemotherapy.

**[0066]** By a "therapeutically efficient amount" is intended an amount of therapeutic agent, an inhibitor of TYR03 tyrosine kinase, administered to a patient that is sufficient to constitute a treatment of bladder cancer as defined above.

**[0067]** The pharmaceutical composition comprising the inhibitor of TYR03 tyrosine kinase is formulated in accordance with standard pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York) known by a person skilled in the art.

**[0068]** Possible pharmaceutical compositions include those suitable for oral, rectal, intravesical, topical (including transdermal, buccal and sublingual), or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. For these formulations, conventional excipient can be used according to techniques well known by those skilled in the art.

**[0069]** The compositions for parenteral administration are generally physiologically compatible sterile solutions or

suspensions which can optionally be prepared immediately before use from solid or lyophilized form. Adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle and a surfactant or wetting agent can be included in the composition to facilitate uniform distribution of the active ingredient.

**[0070]** For oral administration, the composition can be formulated into conventional oral dosage forms such as tablets, capsules, powders, granules and liquid preparations such as syrups, elixirs, and concentrated drops. Non toxic solid carriers or diluents may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. For compressed tablets, binders, which are agents which impart cohesive qualities to powdered materials are also necessary. For example, starch, gelatine, sugars such as lactose or dextrose, and natural or synthetic gums can be used as binders. Disintegrants are also necessary in the tablets to facilitate break-up of the tablet. Disintegrants include starches, clays, celluloses, algin, gums and crosslinked polymers. Moreover, lubricants and glidants are also included in the tablets to prevent adhesion to the tablet material to surfaces in the manufacturing process and to improve the flow characteristics of the powder material during manufacture. Colloidal silicon dioxide is most commonly used as a glidant and compounds such as talc or stearic acids are most commonly used as lubricants.

**[0071]** For transdermal administration, the composition can be formulated into ointment, cream or gel form and appropriate penetrants or detergents could be used to facilitate permeation, such as dimethyl sulfoxide, dimethyl acetamide and dimethylformamide.

**[0072]** For transmucosal administration, nasal sprays, rectal or vaginal suppositories can be used. The active compound can be incorporated into any of the known suppository bases by methods known in the art. Examples of such bases include cocoa butter, polyethylene glycols (carbowaxes), polyethylene sorbitan monostearate, and mixtures of these with other compatible materials to modify the melting point or dissolution rate.

**[0073]** Pharmaceutical compositions according to the invention may be formulated to release the active drug substantially immediately upon administration or at any predetermined time or time period after administration.

**[0074]** Pharmaceutical compositions according to the invention can comprise one or more TYR03 tyrosine kinase inhibitor(s) associated with pharmaceutically acceptable excipients and/or carriers. These excipients and/or carriers are chosen according to the form of administration as described above. Other active compounds can also be associated with TYR03 tyrosine kinase inhibitors such as other molecules used for the treatment of bladder cancer (e.g. cisplatin, adriamycin, mitomycin C, gemcitabine, paclitaxel or docetaxel).

**[0075]** In a particular embodiment, the pharmaceutical composition comprises one or more inhibitor(s) of the TYR03 tyrosine kinase selected from the group consisting of a small molecule inhibiting the tyrosine kinase activity, an antibody directed against the extracellular domain of TYR03, a RNAi molecule specific of TYR03, particularly a siRNA, a dominant negative receptor presenting a kinase dead domain and a TYR03 soluble receptor.

**[0076]** The amount of inhibitor of TYR03 tyrosine kinase to be administered has to be determined by standard procedure well known by those of ordinary skill in the art. Physiological data of the patient (e.g. age, size, and weight), the routes of administration and the disease to be treated have to be taken into account to determine the appropriate dosage.

**[0077]** The inhibitor of TYR03 tyrosine kinase may be administered as a single dose or in multiple doses. If the inhibitor of TYR03 tyrosine kinase is a small molecule inhibiting the tyrosine kinase activity, each unit dosage may contain, for example, from 200 to 1000 mg/kg of body weight, particularly from 500 to 800 mg/kg of body weight. If the inhibitor of TYR03 tyrosine kinase is an antibody directed against the extracellular domain of TYRO3, each unit dosage may contain, for example, from 0.1 to 20 mg/kg of body weight, particularly from 4 to 10 mg/kg of body weight. If the inhibitor of TYR03 tyrosine kinase is a RNAi molecule specific of TYRO3, each unit dosage may contain, for example, from 2 to 50 mg/kg of body weight, particularly from 5 to 20 mg/kg of body weight. If the inhibitor of TYR03 tyrosine kinase is a dominant negative receptor presenting a kinase dead domain or a TYR03 soluble receptor, each unit dosage may contain, for example, from 5 to 100 mg/kg of body weight, particularly from 15 to 70 mg/kg of body weight.

**[0078]** TYR03 inhibitor can be used in combination with other active ingredients, in particular, other TYR03 inhibitors or with other treatments of bladder cancer, such as BCG treatment (e.g. WO05/077411) or administration of anticancer drugs, for example cisplatin, adriamycin, mitomycin C, gemcitabine, paclitaxel or docetaxel. In this case, TYR03 inhibitors and the other molecules can be administered simultaneously or consecutively.

**[0079]** The present invention further provides a method for treating a bladder tumor in a subject comprising administering a therapeutically efficient amount of a TYR03 tyrosine kinase inhibitor to the subject. Preferably, the subject is a human.

**[0080]** The present invention provides a method for screening or identifying a molecule suitable for treating a bladder tumor. This method is based on the analysis of the ability of a molecule to bind to TYR03 receptor, to compete with or for a ligand of TYR03 receptor, to decrease the TYR03 gene expression or to decrease the phosphorylation of the TYR03 substrates or the TYR03 autophosphorylation.

**[0081]** In one embodiment, the method for screening or identifying a molecule suitable for treating a bladder tumor comprises (i) contacting candidate molecules with TYR03 receptor, and (ii) selecting molecules having the ability to bind to TYR03 receptor and/or to compete with and/or for a ligand of TYR03 receptor and/or to decrease the phosphorylation

of the TYR03 substrates or the TYR03 autophosphorylation. The method can comprise a step (i') of determining the ability of candidate molecules to bind to TYR03 receptor and/or to compete with and/or for a ligand of TYR03 receptor and/or to decrease the phosphorylation of the TYR03 substrates or the TYR03 autophosphorylation.

**[0082]** In an other embodiment, the method for screening or identifying a molecule suitable for treating a bladder tumor comprises (i) contacting candidate molecules with cells expressing TYR03 receptor, and (ii) selecting molecules having the ability to bind to TYR03 receptor and/or to compete with and/or for a ligand of TYR03 receptor and/or to decrease the TYR03 gene expression and/or to decrease the phosphorylation of the TYR03 substrates or the TYR03 autophosphorylation. Cells used for this screening can be cells expressing high level of endogenous TYR03, such as most of bladder cell lines, in particular J82 or RT112 cell lines, or genetically modified cells over-expressing TYR03 allowing an optimized detection of tyrosine kinase activity. The method can comprise a step (i') of determining the ability of candidate molecules to bind to TYR03 receptor and/or to compete with and/or for a ligand of TYR03 receptor and/or to decrease the phosphorylation of the TYR03 substrates or the TYR03 autophosphorylation.

**[0083]** The binding of a molecule to TYR03 receptor can be measured by well-known techniques such as surface plasmon resonance, calorimetry or Biacore technology.

**[0084]** The ability of a molecule to compete with or for a ligand of TYR03 receptor can be evaluated, for example, by competition experiments with labelled ligand, in particular radio-labelled ligand, Biacore or spectroscopic observations.

**[0085]** The TYR03 gene expression can be evaluated with different well known techniques, such as quantitative RT-PCR, Northern-blot, ELISA or Western-blot.

**[0086]** The TYR03 phosphorylation level can be assessed by western-blot using an anti-phosphotyrosine or an anti-phospho-TYRO3 antibody, radioactive FlashPlate assay, fluorescent resonance energy transfer (FRET) assay or dissociation-enhance lanthanide fluorescence immunoassay (DELFI).

**[0087]** This method as described above can further comprise a subsequent step consisting of administering previously *in vitro* selected molecule in a bladder tumor non human animal model and analyzing the effect on the tumor progression. The efficiency of the molecule can be evaluated, for instance, by analyzing the life span of animals, the occurrence of metastasis, the progression of the tumor, the occurrence of muscle invasion of cancer. All these characteristics have to be compared with those of controls consisting of bladder tumor non human animal models with no treatment. The non human animal model may be nude mice with grafted tumor, preferably a bladder tumor.

**[0088]** The following examples are given for purposes of illustration and not by way of limitation.

## EXAMPLES

### Example 1: TYRO-3 and GAS6 over-expression in bladder tumors

**[0089]** RNA levels were analyzed using Affymetrix DNA microarrays U95A in 80 bladder carcinomas, 5 normal bladder urothelium. SAM software (<http://www-stat.stanford.edu/~tibs/SAM>) was used to identify genes displaying differential expression between tumoral and normal samples. SAM with the parameters "false discovery rate of 10 %" and "SAM fold change of at least 2" identified 823 probe sets significantly more strongly expressed in tumors as compared to normal urothelium and 477 probe sets less strongly expressed in tumors.

**[0090]** Among these up-regulated genes, TYRO-3 kinase was focused and the results obtained with SAM were confirmed using an ANNOVA test (Figure 1B). The level of TYR03 RNA (MAS 5 Affymetrix DNA chips data) in each tumor sample was then compared with the distribution of TYR03 RNA levels in normal samples and the difference was considered significant if it exceeded three standard deviations (z-score > 3, p < 0.0013). TYR03 was significantly over-expressed in 57/80 tumors (71%). This over-expression was independent of tumor stage and grade (Figure 1A). These results obtained from Affymetrix data were confirmed by Q-RT-PCR analysis (data not shown).

Interestingly, GAS6, the only known ligand of TYR03, was also significantly over-expressed in invasive tumors as compared to normal or superficial tumors (SAM analysis of differentially expressed genes between normal and invasive samples or superficial and invasive tumors, confirmed by ANNOVA test) (Figure 2A).

No correlation between mRNA expression level and DNA copy number at the TYR03 OR GAS6 locus was observed suggesting that TYR03 and GAS6 over-expression were not due do DNA amplification. In situ hybridization showed that TYR03 was expressed by epithelial cells whereas GAS6 is expressed by both epithelial and stromal cells suggesting hence a possible autocrine or paracrine activation of TYR03 by GAS6 in invasive tumors and hence a reinforcement of TYR03 role in those carcinomas (data not shown). No mutation of TYR03 was found in a subset of 15 bladder tumor samples expressing various levels of TYRO3 mRNA.

### Example 2 : Effect of inhibition of TYRO3 activity in bladder tumor cells

**[0091]** To explore the role of TYR03 in bladder carcinoma, the first step was to identify bladder cancer derived cell lines mimicking superficial tumors expressing only TYR03 and invasive tumors expressing both TYR03 and GAS6.

TYR03 and GAS6 mRNA expression levels were therefore investigated in 8 bladder cancer derived cell lines (T24, RT4, KK47, TCCSUP, EJ138, J82 and RT112 cell lines (ATCC) and MGH-U3 cell line (Lin et al., 1985)) and in one normal urothelium derived cell line, NHU (ATCC), by Q-RT-PCR (Figure 3). All studied tumor cell lines expressed more strongly TYR03 as compared to the normal proliferative cell line, suggesting that TYR03 expression was cancer dependent and not linked to cell proliferation.

**[0092]** In order to investigate the role of TYR03 in cell growth and tumorigenic properties, TYR03 expression was blocked using RNA interference technology or TYR03 activity was inhibited using a blocking antibody directed against the extracellular domain of TYR03 or a soluble receptor consisting of the recombinant extracellular domain of TYR03 produced in bacteria. Two cell lines expressing TYR03 (MGH-U3, KK47), two cell lines expressing both TYR03 and GAS6 (J82, RT112) and one control breast cancer derived cell line presenting a very low TYR03 expression level (MCF-7) were used. It was shown by western blot using an anti-phosphotyrosine antibody after TYR03 immunoprecipitation that in each type of cell line (expressing or not GAS6), TYRO was activated (data not shown).

**[0093]** The transfection of MGH-U3, KK47, J82 and RT112 cells with TYR03 siRNAs markedly decreased TYR03 mRNA and protein levels (80- 90 % inhibition) (Results for MGH-U3 cells are presented Figure 4). This knockdown of TYR03 yielded fewer viable MGH-U3, KK47, J82 and RT112 cells than the control siRNA (Figure 5) whereas it had no effect on MCF7 cells, suggesting that the effect observed following transfection with the specific siRNA was rather due to a specific gene silencing than to an off-target effect. The same effect on cell growth was also obtained by blocking TYR03 using a polyclonal antibodies directed against its extra-cellular domain (Figure 6) or a recombinant soluble receptor consisting of the entire extracellular domain of TYR03 (aa 1 to 421) (Figure 7).

**[0094]** This decreased number of viable cells could be attributed to an increased apoptotic rate (Figure 8) with significant but low change in cell-cycle progression (Figure 9). TYR03 knockdown also yielded fewer viable MGH-U3, KK47, RT112 and J82 colonies in soft agar assays demonstrating that TYR03 regulated cell survival of clonogenic cancer cells (Figure 10).

#### Example 3: In vivo studies of the role of TYRO3 in bladder cancer cell growth

**[0095]** Hence our results clearly demonstrated that TYR03 regulate bladder cancer cells survival/proliferation *in vitro*. The role of this gene in bladder cancer cell growth *in vivo* was studied. J82 and MGH-U3 cells were implanted subcutaneously in athymic nude mice. Once tumors were established, mice were randomly selected for treatment with control or TYR03-specific siRNAs. For J82 xenografts, two weeks after the beginning of treatment only three tumors out of 12 were still observed (Figures 11A and 11B). For MGH-U3 xenografts, after 21 days of treatment, tumor volume was 70% lower in mice treated with TYRO3 siRNA than in mice treated with control siRNA (Wilcoxon rank-sum test,  $p < 0.001$ ) (Figures 12A and 12B).

**[0096]** This inhibition of tumor growth was associated with a significant decrease in TYR03 mRNA levels (Student's t-test,  $p < 0.001$ ) measured at a time (3 days after the last siRNA injection) where it should be the lowest one (Figure 13). No histological difference was observed between treated and control tumors (data not shown), but TUNNEL analysis demonstrated that tumor growth inhibition was due to increase apoptosis in treated tumors (data not shown).

**[0097]** Taken together our results identified TYR03 as a major gene implicated in bladder carcinoma being up-regulated in the majority of cases (70-75 % of tumors) independently of tumors stage and/or grade and being responsible for tumor cell survival. Furthermore, these experiments demonstrate that compounds inducing inhibition or depletion of TYRO3 provoke an enhanced apoptosis of bladder tumor cells and, consequently, can be used to treat bladder tumor.

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## SEQUENCE LISTING

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 AP-HP  
 Université Paris XII

<120> Tyrosine kinase receptor TYR03 as a therapeutic target in the  
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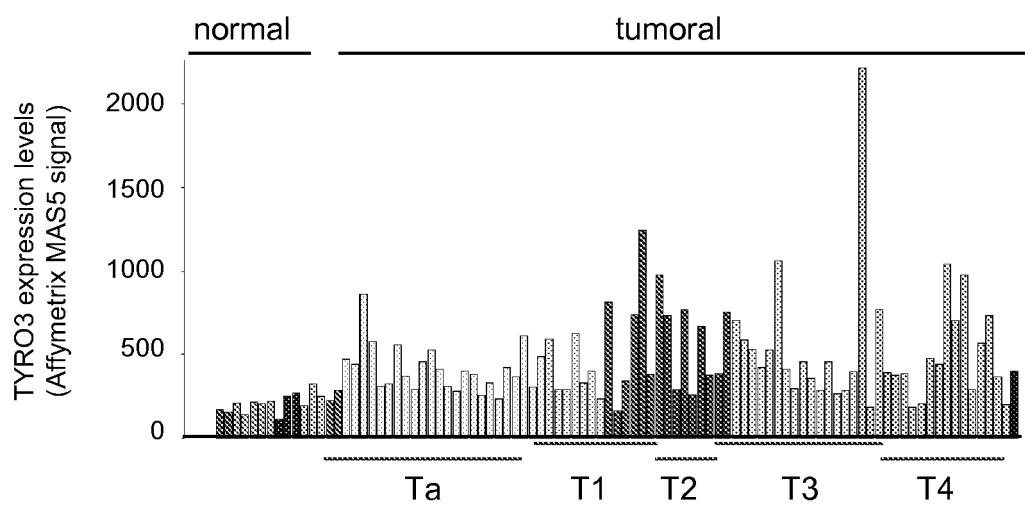
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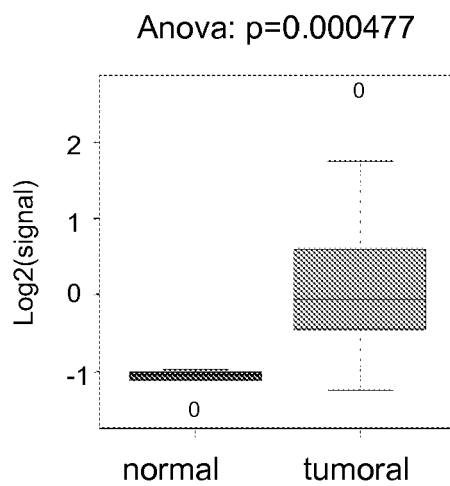
## Claims

- 50 1. Inhibitor of TYR03 tyrosine kinase for the treatment of a bladder tumor.
2. The inhibitor according to claim 1, wherein the inhibitor is selected from the group consisting of a small molecule inhibiting the TYR03 tyrosine kinase activity, an antibody directed against the extracellular domain of TYR03, a nucleic acid molecule interfering specifically with TYR03 expression, a dominant negative receptor presenting a kinase dead domain and a TYR03 soluble bait.
- 55 3. The inhibitor according to claim 2, wherein the nucleic acid molecule interfering specifically with TYR03 expression is a RNAi, an antisense nucleic acid or a ribozyme.

4. The inhibitor according to claim 3, wherein the RNAi is a siRNA, in particular siRNA comprising a sequence of SEQ ID No. 1.
5. The inhibitor according to claim 2, wherein the TYR03 soluble bait is a recombinant TYR03 receptor constituted of, at least, one Ig-like or fibronectin III domain of the extracellular domain of the receptor.
6. The inhibitor according to any one of claims 1 to 5, wherein the inhibitor of TYR03 tyrosine kinase is used in combination with another active ingredient, in particular a bladder tumor treatment.
7. Pharmaceutical composition comprising an inhibitor of TYR03 tyrosine kinase and a pharmaceutically acceptable carrier/excipient for the treatment of a bladder tumor.
8. The pharmaceutical composition according to claim 7, wherein the inhibitor is selected from the group consisting of a small molecule inhibiting the TYR03 tyrosine kinase activity, an antibody directed against the extracellular domain of TYR03, a nucleic acid molecule interfering specifically with TYR03 expression, a dominant negative receptor presenting a kinase dead domain and a TYR03 soluble bait.
9. The pharmaceutical composition according to claim 8, wherein the nucleic acid molecule interfering specifically with TYR03 expression is a RNAi, an antisense nucleic acid or a ribozyme.
10. The pharmaceutical composition according to claim 9, wherein the RNAi is a siRNA.
11. The pharmaceutical composition according to claim 8, wherein the TYR03 soluble bait is a recombinant TYR03 receptor constituted of, at least, one Ig-like or fibronectin III domain of the extracellular domain of the receptor.
12. Method for screening or identifying a molecule suitable for treating a bladder tumor comprises (i) contacting candidate molecules with TYR03 receptor, and (ii) selecting molecules having the ability to bind to TYR03 receptor and/or to compete with and/or for a ligand of TYR03 receptor and/or to decrease the phosphorylation of the TYR03 substrates or the TYR03 autophosphorylation.
13. Method for screening or identifying a molecule suitable for treating a bladder tumor comprising (i) contacting candidate molecules with cells expressing TYR03 receptor, and (ii) selecting molecules having the ability to bind to TYR03 receptor and/or to compete with and/or for a ligand of TYR03 receptor and/or to decrease the TYR03 gene expression and/or to decrease the phosphorylation of the TYR03 substrates or the TYR03 autophosphorylation.
14. The method according to claim 12 or 13, further comprising administering *in vitro* selected molecule in a bladder tumor non human animal model and analyzing the effect on the disease progression.



**Figure 1A**



**Figure 1B**

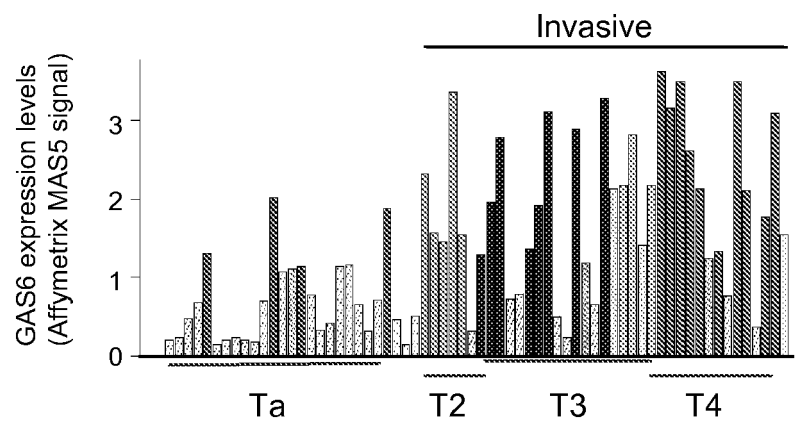


Figure 2A

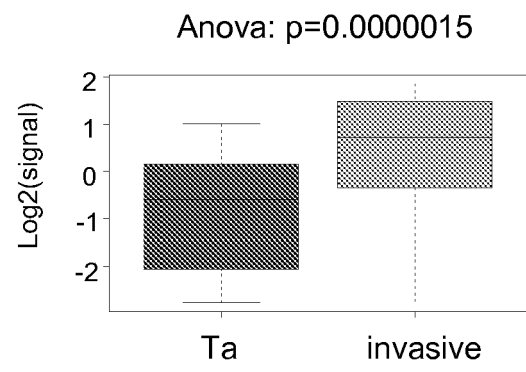


Figure 2B

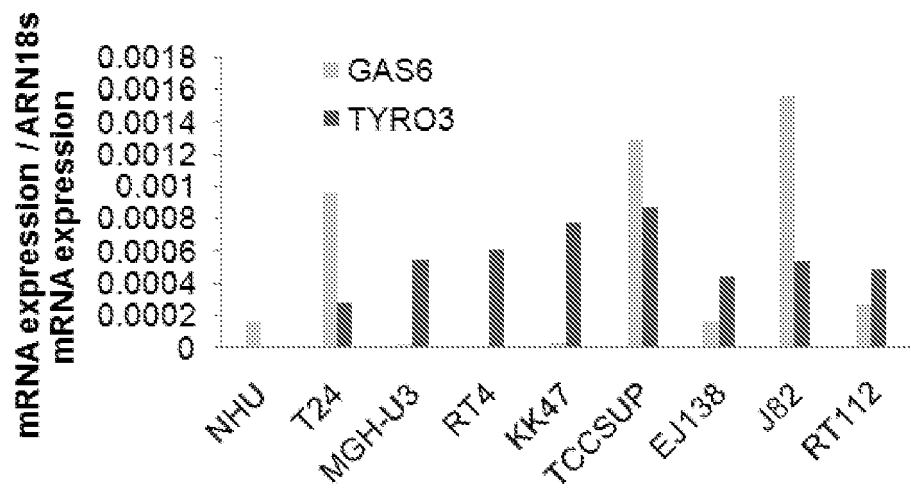


Figure 3

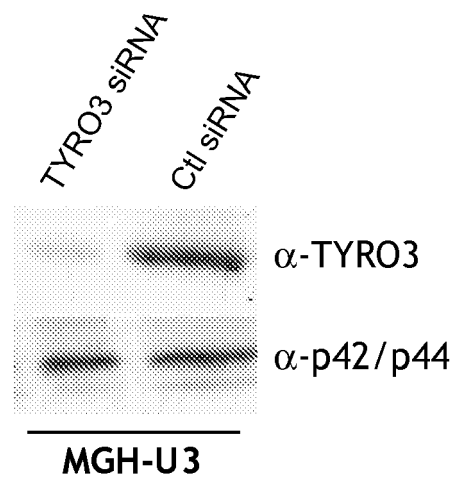


Figure 4

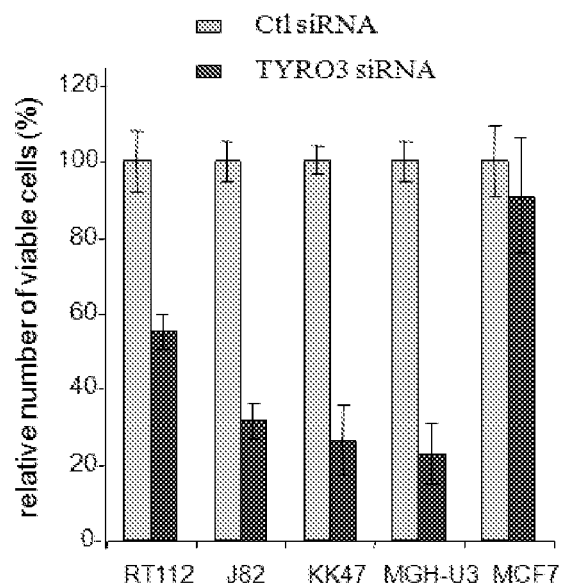


Figure 5

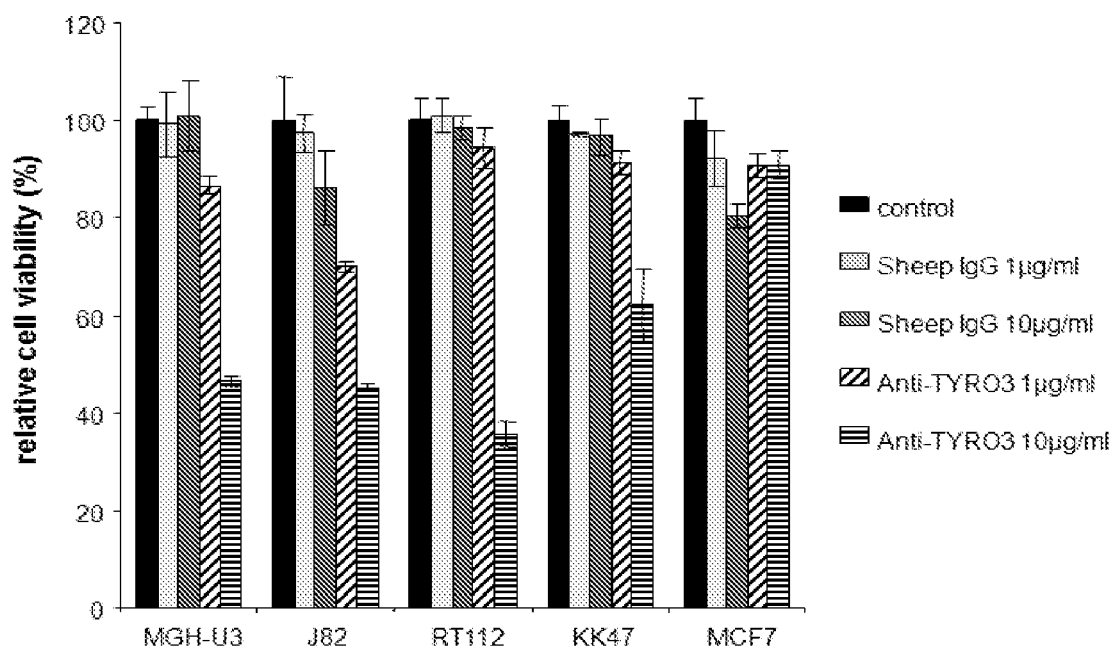


Figure 6

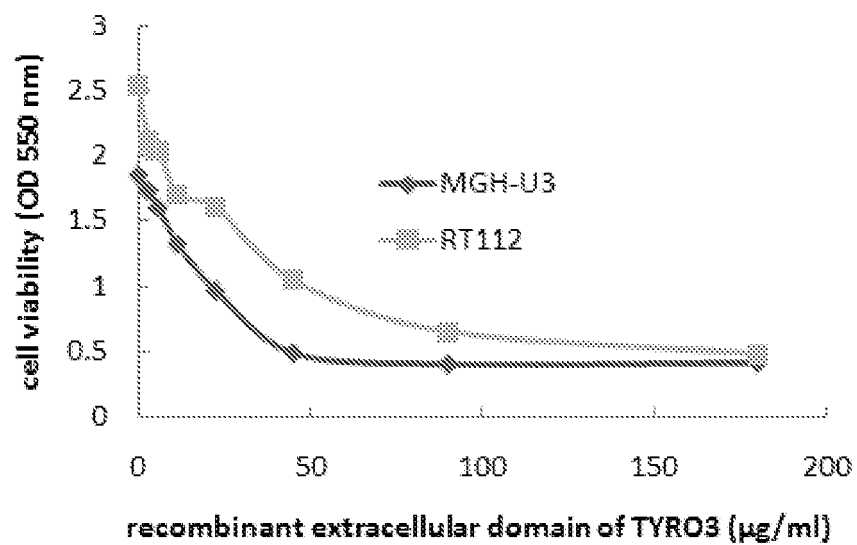


Figure 7

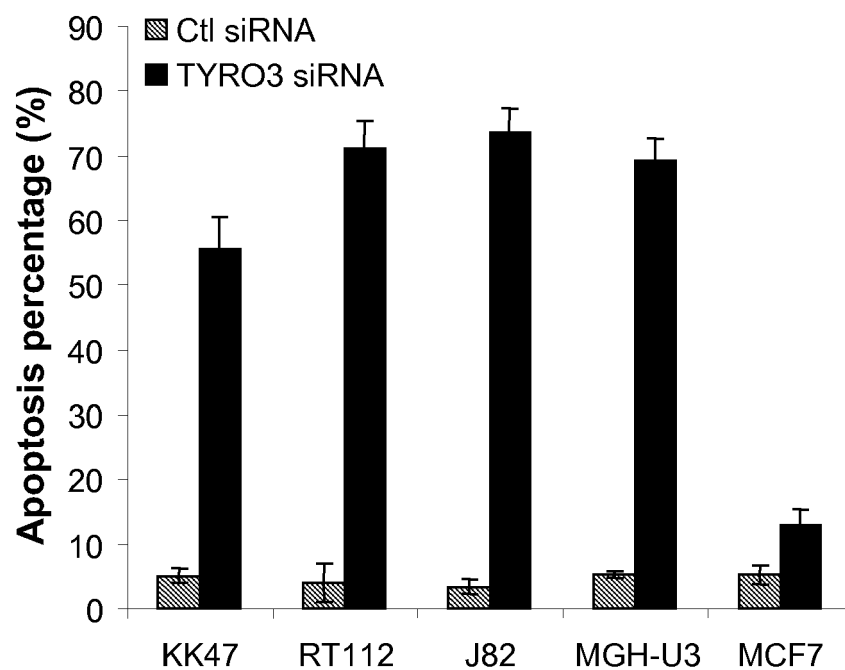


Figure 8

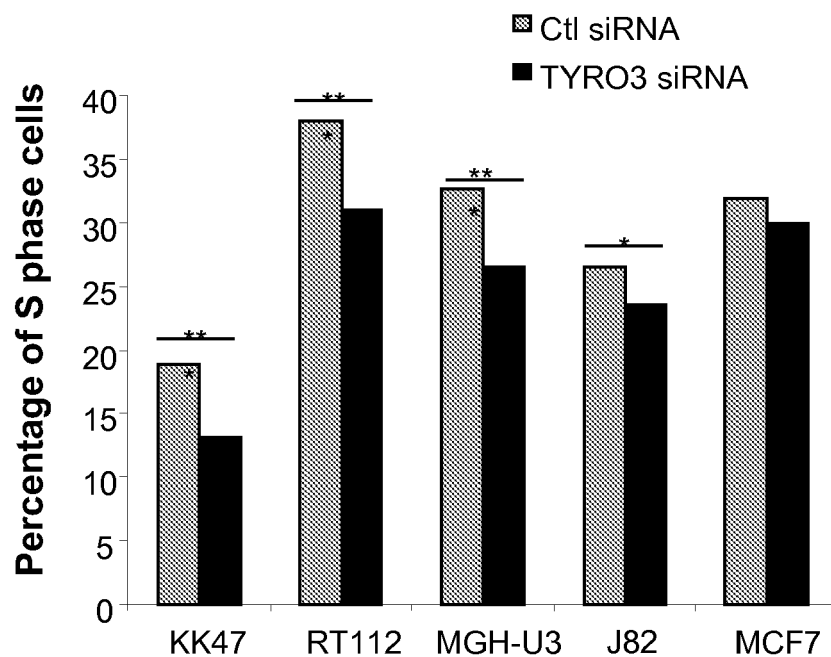


Figure 9

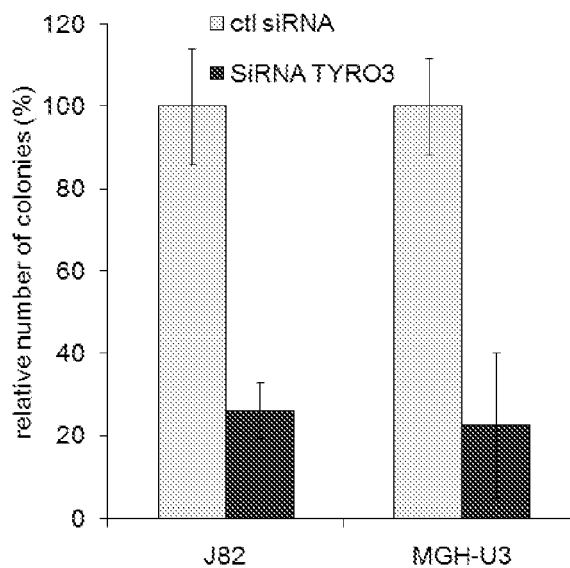
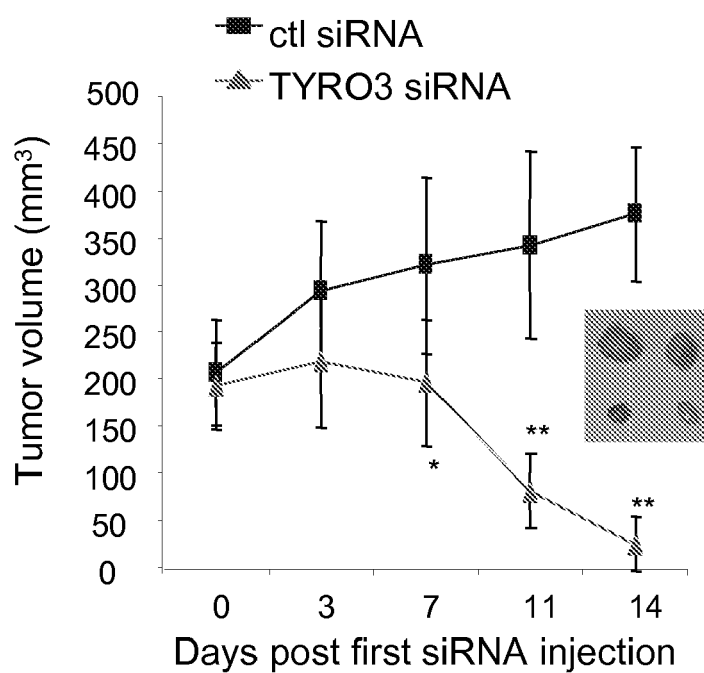
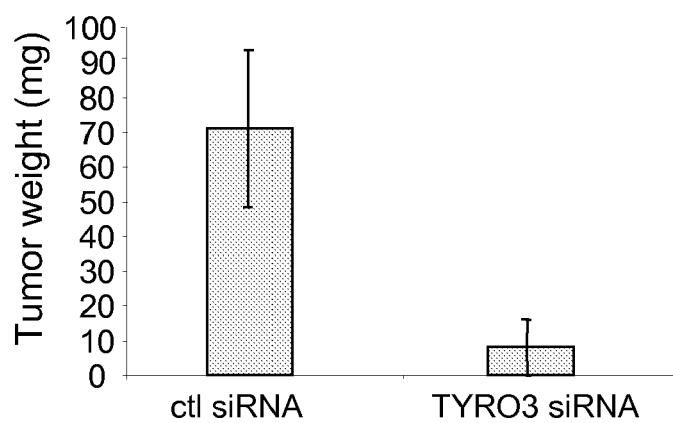


Figure 10



**Figure 11A**



**Figure 11B**

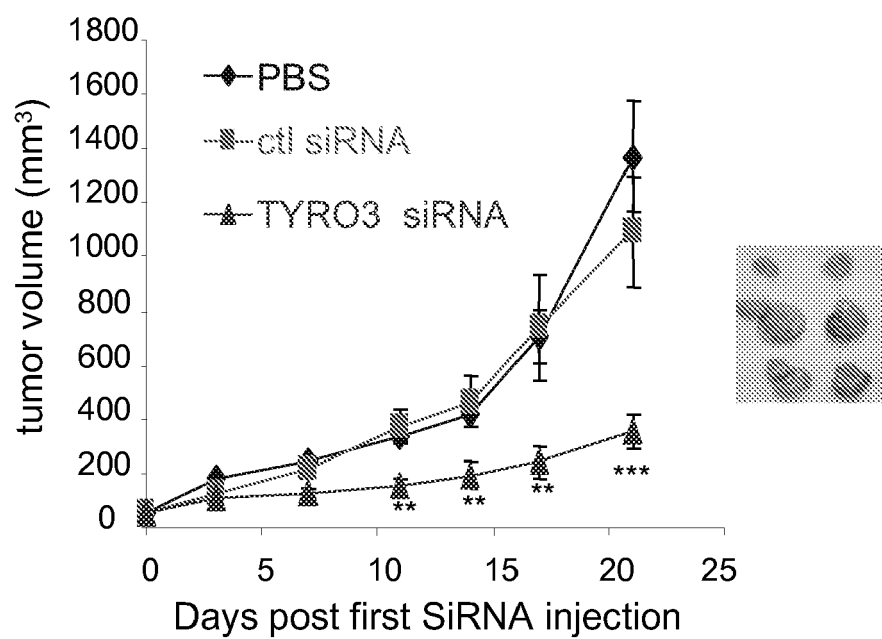


Figure 12A

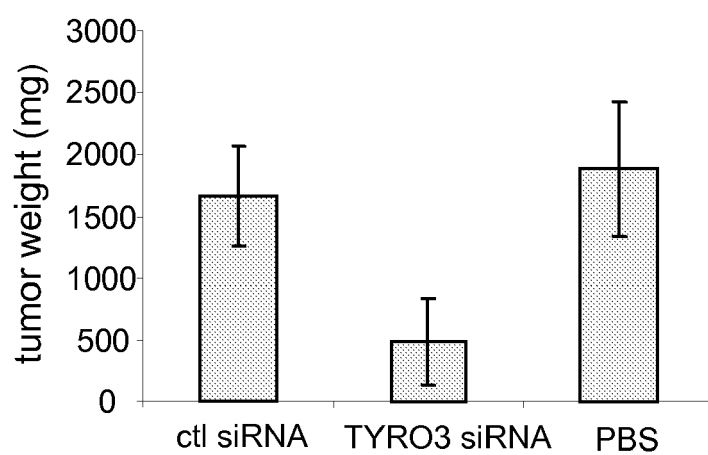
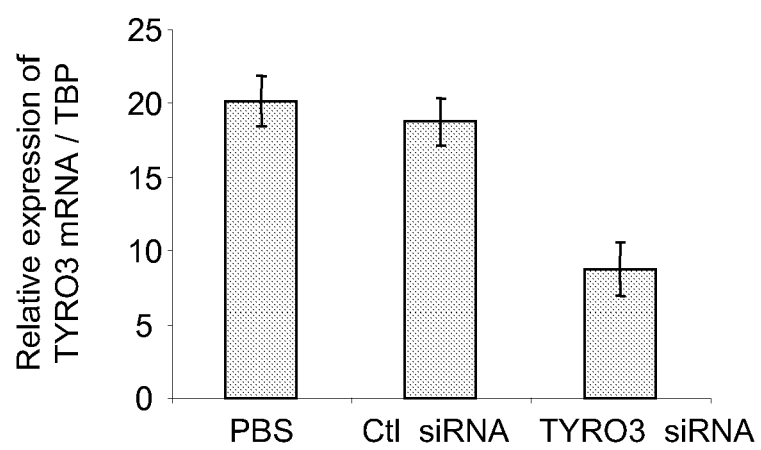


Figure 12B

**Figure 13**



## EUROPEAN SEARCH REPORT

Application Number  
EP 08 30 5574

| DOCUMENTS CONSIDERED TO BE RELEVANT                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                       |                                                                                                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| Category                                                                                                                                                                                                                                                              | Citation of document with indication, where appropriate, of relevant passages                                                                                                                                                                                                                                                                                                                                                                                                      | Relevant to claim                                                                                                                                                                                                                                                                                     | CLASSIFICATION OF THE APPLICATION (IPC)                                                                          |
| X                                                                                                                                                                                                                                                                     | <p>PORTA DIANA GRAUS ET AL: "TK1258, a multi-targeted receptor tyrosine kinase (RTK) inhibitor, is efficacious in preclinical models of bladder cancer." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 49, April 2008 (2008-04), page 1164, XP001537053</p> <p>&amp; 99TH ANNUAL MEETING OF THE AMERICAN-ASSOCIATION-FOR-CANCER-RESEARCH; SAN DIEGO, CA, USA; APRIL 12 -16, 2008<br/>ISSN: 0197-016X<br/>* abstract *</p>                       | 1,2,7,8                                                                                                                                                                                                                                                                                               | <p>INV.<br/>A61K31/7105<br/>A61K38/45<br/>A61K39/395<br/>A61P35/00<br/>A61P13/10<br/>A61K31/47<br/>C12N15/11</p> |
| X                                                                                                                                                                                                                                                                     | <p>KAMAT ASHISH M ET AL: "CI-1033, a pan-erbB tyrosine kinase antagonist, inhibits the growth of orthotopic bladder cancer tumors via direct effects on receptor phosphorylation and inhibition of angiogenesis." JOURNAL OF UROLOGY, vol. 169, no. 4 Supplement, April 2003 (2003-04), pages 130-131, XP009110660</p> <p>&amp; 98TH ANNUAL MEETING OF THE AMERICAN UROLOGICAL ASSOCIATION (AUA); CHICAGO, IL, USA; APRIL 26-MAY 02, 2003<br/>ISSN: 0022-5347<br/>* abstract *</p> | 1,2,7,8                                                                                                                                                                                                                                                                                               | <p>TECHNICAL FIELDS SEARCHED (IPC)</p> <p>A61K<br/>A61P<br/>C12N</p>                                             |
| The present search report has been drawn up for all claims                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                       |                                                                                                                  |
| Place of search<br><b>Munich</b>                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Date of completion of the search<br><b>20 January 2009</b>                                                                                                                                                                                                                                            | Examiner<br><b>Böhmerova, Eva</b>                                                                                |
| <p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone<br/>Y : particularly relevant if combined with another document of the same category<br/>A : technological background<br/>O : non-written disclosure<br/>P : intermediate document</p> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | <p>T : theory or principle underlying the invention<br/>E : earlier patent document, but published on, or after the filing date<br/>D : document cited in the application<br/>L : document cited for other reasons<br/>.....<br/>&amp; : member of the same patent family, corresponding document</p> |                                                                                                                  |

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## EUROPEAN SEARCH REPORT

Application Number  
EP 08 30 5574

| DOCUMENTS CONSIDERED TO BE RELEVANT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                               |                                                     |                                         |
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| Category                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Citation of document with indication, where appropriate, of relevant passages                                                                                                                                                                                                                                                                                                                                 | Relevant to claim                                   | CLASSIFICATION OF THE APPLICATION (IPC) |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | SCHWARTZ G K ET AL: "A PROTEIN KINASE C PKC INHIBITOR STAUROSPORINE STSN INHIBITS INVASION OF CLINICALLY INVASIVE HUMAN BLADDER CARCINOMA CELLS"<br>PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 31, 1990, page 74, XP009110672 & 81ST ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, WASHINGTON, D.C., USA, MAY 23-2<br>ISSN: 0197-016X<br>* abstract * | 1,2,7,8                                             |                                         |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | PEARSON ET AL: "A PHASE II STUDY OF NEOADJUVANT ERLOTINIB (TARCEVA) IN PATIENTS WITH MUSCLE-INVASIVE BLADDER CANCER UNDERGOING RADICAL CYSTECTOMY: PRELIMINARY RESULTS"<br>JOURNAL OF UROLOGY, BALTIMORE, MD, US, vol. 179, no. 4, 19 March 2008 (2008-03-19), page 119, XP022543376<br>ISSN: 0022-5347<br>* abstract *                                                                                       | 1,2,7,8                                             |                                         |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | SILAY ET AL: "Sunitinib malate and sorafenib may be beneficial at the treatment of advanced bladder cancer due to their anti-angiogenic effects"<br>MEDICAL HYPOTHESES, EDEN PRESS, PENRITH, US, vol. 69, no. 4, 1 January 2007 (2007-01-01), pages 892-895, XP022205174<br>ISSN: 0306-9877<br>* the whole document *                                                                                         | 1,2,7,8                                             |                                         |
| The present search report has been drawn up for all claims                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                               |                                                     | TECHNICAL FIELDS SEARCHED (IPC)         |
| 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Place of search<br>Munich                                                                                                                                                                                                                                                                                                                                                                                     | Date of completion of the search<br>20 January 2009 | Examiner<br>Böhmerova, Eva              |
| <p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone<br/>Y : particularly relevant if combined with another document of the same category<br/>A : technological background<br/>O : non-written disclosure<br/>P : intermediate document</p> <p>T : theory or principle underlying the invention<br/>E : earlier patent document, but published on, or after the filing date<br/>D : document cited in the application<br/>L : document cited for other reasons<br/>&amp; : member of the same patent family, corresponding document</p> |                                                                                                                                                                                                                                                                                                                                                                                                               |                                                     |                                         |

EPO FORM 1503 03.82 (P04C01)



## EUROPEAN SEARCH REPORT

Application Number  
EP 08 30 5574

| DOCUMENTS CONSIDERED TO BE RELEVANT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                                                                                                                                           |                                                     |                                         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------|-----------------------------------------|
| Category                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Citation of document with indication, where appropriate, of relevant passages                                                                                                                                                             | Relevant to claim                                   | CLASSIFICATION OF THE APPLICATION (IPC) |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | AGARWAL P K ET AL: "Emerging drugs for targeted therapy of bladder cancer"<br>EXPERT OPINION ON EMERGING DRUGS 200709 GB,<br>vol. 12, no. 3, September 2007 (2007-09),<br>pages 435-448, XP009110654<br>ISSN: 1472-8214<br>* tables 1,2 * | 1,2,7,8                                             |                                         |
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| A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | -----<br>WO 2008/051808 A (SGX PHARMACEUTICALS INC [US]; BOUNAUD PIERRE-YVES [US]; SMITH CHRISTOP) 2 May 2008 (2008-05-02)<br>* paragraphs [0007], [0008], [0179], [0180], [0182], [0183]; claims 19-23 *                                 | 1-14                                                |                                         |
| D,A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | -----<br>WO 2005/000207 A (MEDIMMUNE INC [US]; KIENER PETER [US]; LANGERMANN SOLOMON [US]; KINCH) 6 January 2005 (2005-01-06)<br>*claims*<br>* page 5, line 15 - page 6, line 25 *                                                        | 1-14                                                |                                         |
| The present search report has been drawn up for all claims                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                           |                                                     | TECHNICAL FIELDS SEARCHED (IPC)         |
| Place of search<br>Munich                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                           | Date of completion of the search<br>20 January 2009 | Examiner<br>Böhmerova, Eva              |
| <p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone<br/>Y : particularly relevant if combined with another document of the same category<br/>A : technological background<br/>O : non-written disclosure<br/>P : intermediate document</p> <p>T : theory or principle underlying the invention<br/>E : earlier patent document, but published on, or after the filing date<br/>D : document cited in the application<br/>L : document cited for other reasons<br/>&amp; : member of the same patent family, corresponding document</p> |                                                                                                                                                                                                                                           |                                                     |                                         |

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**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 08 30 5574

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

20-01-2009

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